opening or closure of the channel gate through an electromechanical coupling. In other channels a direct interaction between the S4-S5 linker and bottom part of S6 has been shown to constitute this electromechanical coupling. We previously identified residues in the C-terminal part of S6 that are critical for KCNQ1 gating. To investigate if these residues interact with the S4-S5 linker, we performed an alanine/tryptophan substitution scan of the S4-S5 linker sequence. Based on their impact on channel gating, we categorized these substitutions as either ''high'' or ''low impact''. The pattern of ''high impact'' positions was consistent with an α-helical configuration and clustered on one side of the S4-S5 linker. Since substitutions at these positions markedly impaired channel gating, they are good candidates to contact residues in the bottom part of S6. Indeed, replacing valine 254 in the S4-S5 linker by a leucine resulted in channels that were partially constitutively open but channel closure could be rescued by combining V254L with the S6 mutation L353A that by itself displayed a similar phenotype as V254L. The observation that all known LQT1 mutations in the S4-S5 linker map on the ''high impact'' side further strengthens the proposal that this face of the S4-S5 linker contacts the C-terminal S6 segment and constitutes part of the electromechanical coupling in KCNQ1 channels.

631-Pos

Estimating Conformational Changes of KCNQ1 Channels During Gating Using Molecular Dynamics Simulations

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Although the structure of an ion-channel protein may be known through crystallography or homology modeling, measuring its conformational changes during gating is extremely difficult if not impossible. Alternatively, molecular simulations is a powerful tool for estimating conformational changes. Movement of the positively charged S4 segment is a known conformational change of voltage-gated ion-channels, observed by measuring the accessibility of different amino-acid residues.

A previously determined structure of KCNQ1 was used. Movement of the S4-S3 complex with 3 translational and 2 rotational degrees of freedom was assumed to be the major conformational change during gating. More than 1 million conformations were considered. This expanded configuration space (compared to 1 translational and 1 rotational degree of freedom and about 2000 conformations used previously) enabled more accurate analysis of stable conformations. Conformations with steric overlap were eliminated. The electrostatic energies of the remaining conformations for various membrane potentials were computed and used to determine the probability of the ion-channel residing in each conformation (state residency). Conformations with small state residencies were eliminated.

Two regions in the configuration space with high state residency were identified: one associated with open conformations (outward position of S4-S3) and the other with closed conformations (inward position of S4-S3). These regions were separated by a narrow region with low state residency (an energy barrier). The open state consists of a large number of conformations while the energy barrier and its adjacent closed state (intermediate closed) consist of only few conformations. Channel conformations may branch from the intermediate closed conformation into two trajectories toward different subsets of closed conformations (deep closed). The simulated steady state open probabilities at various membrane potentials were consistent with experimental channel activation curves.

632-Pos

Neighboring Alpha-Subunit (KCNQ1) Mutations with a Gain-of-Function IKs Phenotype Show Differential Dependence on Presence of Beta-Subunit (KCNE1)

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The IKs cardiac potassium channel forms through co-assembly of KCNQ1, a 6 transmembrane-(TM) spanning voltage-gated potassium channel α subunit and KCNE1, a single TM spanning accessory protein. Two mutations in the S1 transmembrane helix of KCNQ1, S140G and V141M, have been shown to cause a hyperpolarizing shift in the voltage dependence of channel activation and to disrupt deactivation, resulting in accumulation of open channels and a gain-of-function phenotype during repetitive activity that is causally related to congenital human atrial fibrillation. Initial reports suggested that the phenotype of these mutants depends on the presence of the accessory protein KCNE1, which has been shown to be close in proximity to KCNQ1 S1, raising the possibility that KCNE1 directly interacts with KCNQ1 position 140 and/or 141. Here, we show that a Cys substituted at KCNQ1 position 141 spontaneously crosslinks with cysteines introduced in two positions in KCNE1, but a Cys substituted at position 140 does not crosslink to any Cys-substituted KCNE1 residues tested. Co-expression of KCNE1 with either S140G or V141M KCNQ1 slows deactivation and causes similar negative shifts in channel activation. However, in whole-cell patch clamp experiments using isotonic potassium to explore channel deactivation across a wide range of hyperpolarized potentials, we find that the V141M channel activity is indistinguishable from WT while the S140G mutation shifts the V1/2 of activation -30mV and drastically slows deactivation (tau ~1500ms vs. ~150ms) when compared with wild-type KCNQ1. Taken together, our results support: 1) an orientation in which KCNQ1 residue V141, but not S140, points toward and is in close proximity to KCNE1 and 2) a direct effect of S140G on channel gating but an allosteric effect of V141M on channel gating that requires the presence of KCNE1.

633-Pos

Physical and Functional Interactions Between the KCNQ1 and KCNE1 C-Terminal Domains

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KCNE1 is a regulatory subunit that associates with pore-forming subunits of KCNQ1 to form a channel complex that carries the slowly activating delayed rectifier current, IKs. Interactions between the transmembrane regions of KCNE1 and KCNQ1 S6 control activation kinetics of IKs, while interactions between the intracellular C-termini of the two subunits may dictate deactivation kinetics. Numerous Long QT Syndrome mutations occur in the C-termini of KCNQ1 and KCNE1, indicating the functional importance of these regions. We have located the proximal third of the KCNQ1 C-terminus (KCNQ1-CT) as a site of direct interaction with the KCNE1 C-terminus (KCNE1-CT) via co-immunoprecipitation studies, in vitro pull-down assays and Surface Plasmon Resonance analyses of purified recombinant proteins. Electrophysiological studies employing co-expressed soluble KCNE1-CT with full length KCNQ1 now provide functional evidence that support the physical association findings. When KCNE1-CT is co-expressed with KCNQ1 in CHO cells and analyzed by whole-cell patch clamp, deactivation kinetics of the KCNQ1 current are accelerated. Similarly, deactivation of IKs is accelerated by co-expressing KCNE1-CT with KCNQ1 and full-length KCNE1. KCNE1-CT also shifts the voltage dependence of activation of KCNQ1 current but not IKs current, and has no significant effect on activation kinetics of either current. Thus, excess soluble KCNE1-CT is capable of interacting with KCNQ1 and can perturb the KCNQ1/KCNE1-C-terminal interactions determining deactivation rates of IKs. Work in the laboratory now focuses on identifying the interacting residues of the KCNQ1 and KCNE1 C-termini. Preliminary experiments using Hydrogen-Deuterium Exchange coupled to Mass Spectrometry show that deuterium incorporation into purified KCNE1-CT is slowed by the addition of KCNQ1-CT, implying that binding between the two peptides protect certain residues of KCNE1-CT from being deuterated. Further experiments will work towards delineating the precise structural nature of this interaction.

634-Pos

Partial Restoration of the Cardiac KCNQ1 Mutant A341V by the KCNE1 Auxiliary Subunit

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Dysfunction of the cardiac potassium channel, IKs, can lead to the prolongation of the QT interval in the electrocardiogram, a potentially life-threatening condition known as the long QT syndrome (LQTS). The IKs consists of a pore-forming subunit, encoded by KCNQ1, and an auxiliary subunit, encoded by KCNE1. One of the most common mutations associated with the inherited form of LQTS is the substitution of an alanine residue to a valine at position 341 in KCNQ1. In the present study, we investigated the impact of the KCNE1 subunit on the A341V mutant. The whole-cell patch clamp technique was used to record current from transiently transfected HL-1 cells, a cardiac cell line derived from mouse atrial cells. In contrast to the non-functional A341V, the cotransfection of A341V with KCNE1 (A341V+KCNE1) resulted in a functional channel. Though the resultant current was qualitatively similar to that of native IKs, A341V+KCNE1 resulted in a significantly smaller mean current density, longer time to half-activation and a rightward shifted voltage dependence of activation with a steeper slope than the wild-type KCNQ1+KCNE1. To determine whether the observed functional restoration of A341V by KCNE1 involved trafficking of the mutant construct, HL-1 cells were transfected with a GFP-tagged A341V with and without KCNE1 and visualized by confocal microscopy. No differences

in the expression pattern of A341V in the absence or presence of KCNE1 were observed. Thus, the partial functional restoration by KCNE1 was not due to trafficking of A341V to the sarcolemma. To confirm that KCNE1 functionally rescued A341V, a mutant KCNE1, T58A that prevents the interaction of KCNE1 with KCNQ1, was cotransfected with A341V. T58A was unable to functionally restore A341V. Our studies showed an intriguing ability of KCNE1 to functionally rescue an LQTS-associated KCNQ1 mutant.

635-Pos

Differential Molecular Motions of KCNE Subunits in the I_{Ks} Channel Complex Detected by Substituted Cysteine-Accessibility Test and Disulfide-Trapping Experiments

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The I_{Ks} channel consists of a pore-forming KCNQ1 channel and auxiliary KCNE subunits: KCNE1 as the major determinant of I_{Ks} gating kinetics while KCNE2 as a regulator of I_{Ks} current amplitude. Knowledge about the structural basis of KCNQ1 modulation by these different KCNE subunits can help the design of KCNE-specific I_{Ks} modulators as antiarrhythmic agents. Recent studies showed that the extracellular-juxtamembranous region of KCNE1 could adopt highly flexible structures and make gating state-dependent contacts with the voltage-sensing domain (VSD) and pore-domain (PD) of KCNQ1. KCNQ1-KCNE1 interactions in this extracellular-juxtamembranous region are critical for the I_{Ks} channel function, as evidenced by congenital arrhythmia-associated mutations identified in this region. Whether KCNE2 can do the same is unclear. We systematically probe the functional role of the extracellular-juxtamembranous region of KCNE1 (positions 36-47) and KCNE2 (positions 39-50), using cysteine-scanning mutagenesis followed by cysteine-accessibility test and disulfide-trapping experiments. MTSET modification of KCNE1 40C-46C alters the voltage-dependence of I_{Ks} activation and, for 44C-46C, reduces the current amplitude. There is a gradient in MTSET modification rates, from fast-reacting (41C-42C) to slow-reacting (44C-46C), with 43C reporting state-dependent accessibility: fast-reacting in open-state & slow-reacting in closed-state. While extensive disulfide-bond partners are found between KCNE1 36C-43C and KCNQ1 144C-147C, no such disulfide-bond partners can be identified between the equivalent KCNE2 positions and KCNQ1 140C-148C. We propose that the KCNE transmembrane helices reside in the junction between VSDs and PD of the KCNQ1 channel, with a similar orientation (with respect to KCNQ1) and position (with respect to membrane bilayer). However, their extracellular-juxtamembranous regions can make differential contacts with KCNQ1, that contribute to their differential effects on the KCNQ1 channel function and provide an opportunity for the design of KCNE-specific I_{Ks} modulators.

636-Pos

Ether ȁ go-go Potassium Channels KCNH1 and KCNH5 Have Four Functional Orthologs in Danio Rerio

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The human ether à go-go channels KCNH1 and KCNH5 form a subgroup with unique functional properties within the eight-membered KCNH family of voltage-gated potassium channels. In mammals, the expression of both genes is mainly restricted to the brain, implying a role in electrical signaling of neurons. Heterologous expression in mammalian cells and *Xenopus* oocytes revealed that ether à go-go channels are sensitive to intracellular Ca^{2+}/calmodulin. The second functional characteristic of both channels is a pronounced slowing of activation kinetics upon binding of extracellular divalent cations. To elucidate the physiological relevance of such properties, an adequate model organism would be desirable. Here we studied the expression of ether à go-go orthologs in zebrafish. Due to a whole-genome duplication during evolution of teleost fish, mammalian genes can have two functional orthologs in zebrafish. However, for the majority of duplicated genes, one gene copy is lost or present as nonfunctional pseudogene only. Using *in silico* screening of genome databases and cloning from reverse transcribed mRNA, we could show that for each of the two human ether à go-go channels two functional orthologs exist in zebrafish. Upon expression in *Xenopus* oocytes, all four genes generated functional channels with current-voltage relationships similar to the human orthologs, characterized by a very negative threshold of the activation voltages. A more pronounced slowing of activation kinetics in the presence of extracellular Mg^{2+} ions clearly distinguished KCNH5 and its two fish orthologs from human KCNH1 and the corresponding fish channels. In summary, the structural and functional conservation between human and fish *ether à go-go* channels makes zebrafish a promising model, but gene duplication must be taken into account. This may also be relevant for the closely related paralogs ERG and ELK.

637-Pos

S4 Arginines Make Unique Contributions to Voltage Dependent Gating Due to Electrostatic Interactions and the Membrane Potential

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Conserved positively charged arginines in the fourth transmembrane segment (S4) of Kv channels are responsible for imparting voltage sensitivity to the channel. There are several forces that may influence these arginines including the membrane potential and electrostatic interactions with countercharges. In Shaker channels, the first four arginines are the primary gating charges that sense the membrane potential. Kv7.1 has fewer positively charged S4 residues than Shaker, notably with the third arginine in Shaker replaced by a glutamine (Q3). Further loss of charge induced by charge reversal at R1 (R1E) in Kv7.1 results in constitutively activated channels, perhaps due to insufficient charge in S4. Consistent with this idea, introduction of a positive charge at Q3 (Q3R) can restore voltage dependent activation to R1E, suggesting that Q3R may substitute for the loss of gating charge at R1E. In a related study, we have demonstrated in Kv7.1 channels that residues corresponding to the first four arginines in Shaker channels (R1-R4) interact sequentially with the first conserved glutamate in S2 (E1) during gating. Here we show via intragenic suppression that S4 arginines also interact electrostatically with the second conserved glutamate in S2 (E2), and these electrostatic interactions play an important role in voltage sensing of S4. Therefore, a network of electrostatic interactions and the membrane potential act on S4 arginines, and the balance of these forces stabilize the conformation of the voltage sensor at different states. The combination of these interactions acts uniquely on each arginine such that each arginine plays a different role in voltage dependent gating. In Kv7.1, the first two arginines (R1, R2) stabilize the resting state while the last three charged residues (R4, H5, R6) stabilize the activated state.

638-Pos

Non-Toxin Gating Modifiers Reveal the Promiscuous Nature of the Voltage Sensor of Kv7.2 and TRPV1 Channels

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Voltage-sensitive cation channel architecture consists of two main structural modules, the voltage sensor domain (VSD) and the pore domain. The VSDs are the target of various gating-modifier toxins and their paddle motifs are modular and transferable structures. Here we show that NH17 and NH29, two new Kv7.2 channel blocker and opener, respectively, act as non-toxin gating modifiers. Mutagenesis and modeling data suggest that in Kv7.2, NH29 docks to the external groove formed by the interface of helices S1, S2 and S4 in a way, which stabilizes the interaction between two conserved charged residues in S2, and S4, known to interact electrostatically, in the open state of Kv channels. Reflecting the promiscuous nature of the VSD, NH29 is also a potent blocker of TRPV1 channels, a feature similar to that described for tarantula toxins. TRPV1 channels appear to be weakly voltage-dependent. However, NH29 changes the linear TRPV1 current-voltage relation obtained with capsaicin, to an outwardly rectifying shape. Interestingly, mutations in linker S3-S4 of the TRPV1 VSD are significantly more resistant to the inhibitory effect of NH29. While compound NH17 potently blocks Kv7.2 channels, it sensitizes the TRPV1 current activated by capsaicin. Mutations in linker S3-S4 switch the TRPV1 sensitizing action of NH17 to a potent inhibition. Subtle modifications in the VSD or in the chemical structure of the molecule drastically change the attributes of the gating-modifier, thereby stabilizing the channel in either the closed or the open state. Data indicate that the novel compounds may operate via a voltage-sensor trapping mechanism similar to that suggested for scorpion and sea anemone toxins. Thus, the VSDs of Kv and TRPV1 channels are promiscuous and share some common structural and biophysical features.

639-Pos

Molecular Mechanism of Slow Kv7.1 Inactivation

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The molecular mechanisms involved in slow C-type inactivation are not clear yet but may involve structural rearrangements in the outer pore domain